Day 9 Worksheet – MACS
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MACS https://github.com/macs3-project/MACS

Introduction: To study DNA enrichment assays such as ChIP-seq and ATAC-seq, we are introducing the analysis method, **M**odel-based **A**nalysis of **C**hIP-**S**eq (MACS). This method enables us to identify transcription factor binding sites and significant DNA read coverage through a combination of gene orientation and sequencing tag position.

! Note: The directory and username used in the screenshot will be for my working directory and username and will be different than yours.

Make working directories
Similar to previous worksheets, make the necessary working directories for running MACS. Repeat the same process for running MACS.

1. Use command **pwd** to determine what directory you are in and if necessary, **cd** to the directory that you want to place your new macs directory in.
2. Make a new directory using the **mkdir** command. Use command **ls -lsh** to confirm the folders are present.

```
-bash-4.2$ mkdir macs2 macs2/output macs2/scripts
```

MACS
3. Pull from the git repo > day9 the **d9_macs.sbatch** from the sample script folder into your scratch directory that you made in the previous exercise. Recall to copy the script, the command syntax is **rsync <input><output>** or **cp.**

4. Edit the sbatch script by using **vim <sbatch>** to open a text editor on your sbatch script. Type **i** to toggle into edit/insert mode. Similar to the previous exercise you will need to change the job name, user email, and the standard output and error log directories. Change the **–job-name=<JOB_NAME>** to a name related to the job you will be running, for example 'trim_qc'. Additionally you will want to change the **–mail-user=<YOUR_EMAIL>** to your email, as well as the path to your eofiles directory for the standard output (**--output)** and error log (**--error)**. The **%x** will be replaced by your **-job-name** and the **%j** will be replace by the job id that will be assigned by the job manager when you run your sbatch script.

```
#!/bin/bash
#SBATCH --job-name=macs2                                    # Job name
#SBATCH --mail-type=ALL                                     # Mail events (NONE, BEG
IN, END, FAIL, ALL)
#SBATCH --mail-user=<USER@EMAIL.COM>                        # Where to send mail
#SBATCH --nodes=1                                           # Numbers of nodes
#SBATCH --ntasks=1                                          # Number of CPU (tasks)
#SBATCH --time=00:30:00                                     # Time limit hrs:min:sec
#SBATCH --partition=compute                                 # Partition/queue reques
ted on server
#SBATCH --mem=2gb                                           # Memory limit
#SBATCH --output=/path/to/eofiles/%x_%j.out
#SBATCH --error=/path/to/eofiles/%x_%j.err
```

5. **module load**. To run MACS, we will need to load python since MACS is dependent on it. In addition we will want to load bedtools which we will use later to remove Blacklist regions.

```
################### LOAD MODULES ###################################

module load python/2.7.14
module load python/2.7.14/MACS/2.1.1
module load bedtools/2.25.0
```

6. **Set variable**. Assigning variables will make your scripts easier to read. In addition, this makes it easier to reference to a given path and utilize it in your scripts.

For the **INDIR=**change the path to the bam files directory. We will be using bam file from ChIP-seq data that used a specific transcription factor. For the **OUTDIR=**, point to the appropriate output file directories for our MACS output files. You can use the command **mkdir -p** just in case for my output directories if you want to ensure that the output directory exist.

In addition, I have uploaded a file of blacklist regions in day9 data directory. These are regions that have been identified as having unstructured or high signal in Nextgen sequencing experiment independent of the cell line or experiment. Removing these will clean up our genomic data for improved quality measurement. ENCODE has a defined list. The list we are using comes from the following reference: Amemiya HM, Kundaje A, Boyle AP. The ENCODE blacklist: identification of problematic regions of the genome. Sci Rep. 2019 Dec; 9(1) 9354 DOI: 10.1038/s41598-019-45839-z

Lastly, I am using the variable **FILENAME** so that I can quickly interchange different files for analysis and only have to change the variable rather than go through my script to change instances of the file.

```
################## SET VARIABLES ###################################

#INDIR is where my bams file are stored. OUTDIR is where I want my output from
running MACS to go
INDIR='/path/to/bam/file'
OUTDIR='/path/to/output/diretory'

BLACKLIST='/scratch/Shares/dowell/sread/data_files/day9/blacklist_hg38/problema
tic_regions_hg38.bed'

FILENAME='BACH1'
```

7. To run the MACS program, we have many different subcommand options. Depending on your experiment, you will want to change the subcommands to fit your requirement.

Usage

```
macs2 [-h] [--version]
    {callpeak,bdgpeakcall,bdgbroadcall,bdgcmp,bdgopt,cmbreps,bdgdiff,filterdup,predictd,pileup
```

Example for regular peak calling: `macs2 callpeak -t ChIP.bam -c Control.bam -f BAM -g hs -n test -B -q 0.01`

Example for broad peak calling: `macs2 callpeak -t ChIP.bam -c Control.bam --broad -g hs --broad-cutoff 0.1`

There are twelve functions available in MAC2S serving as sub-commands.

Reference: https://pypi.org/project/MACS2/

For today's worksheet, we will be showing an example where we utilized an input control with your experiment.

-t / --treatment <filename> is your experimental file. The file can be in any supported format (see –format for options). If you have more than one alignment file, you can specify them and MACS will pool all the files together.

-c / --control <filename> is your genomic input/control file.

-n / --name <NAME> is the name string of your experiment. The string **NAME** will be used by MACS to create output files.

-B/ --BDG flag to tell MACS to strore the fragment fileup, control lambda in bedGraph files.

-g / --gsize <GENOME> is the parameter to assign the mappable genome size. We will be using hs which is the recommended human genome size of 2.7e9.

-q / --qvalue <VALUE> is the cutoff to call significant regions. The default is 0.05. If you want to use a p-value cutoff, you can specify **-p** instead of **-q**.

Note that there are many other options then the ones that we are implementing here.

```
echo macs2
date
date

#### Call peaks with controls
macs2 callpeak \
 -c ${INDIR}/${FILENAME}.input.chr21.sorted.bam \
 -t ${INDIR}/${FILENAME}.chr21.sorted.bam \
 --outdir ${OUTDIR} \
 -n ${FILENAME} \
 -g hs \
 -B \
 -q 0.01 \
```

If you wanted to run to get Broad peaks you will want to use the flag **–broad.**

MACS parameter depending on datatype recommendations

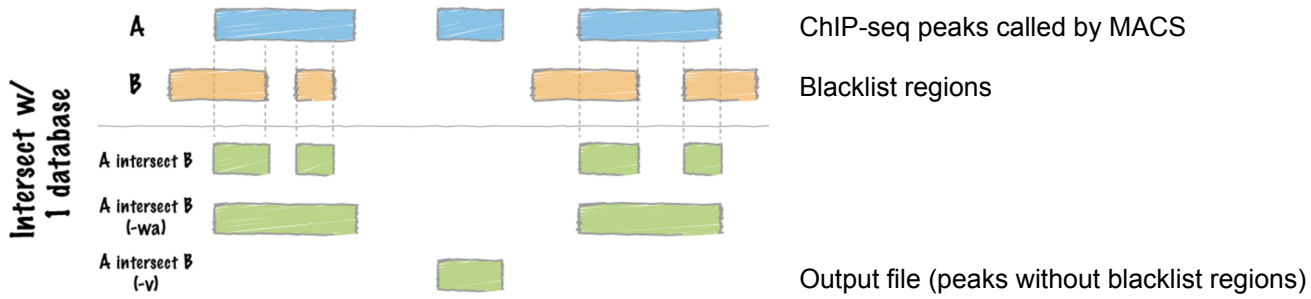
Data type	**q value**	**--broad and --control flags**	**Reasoning**
ChIP-seq for TF	<0.01	--control <INPUT>	TF ChIP-seq often has very abrupt, small peaks that are well defined, so narrow peaks is necessary, and a less stringent adjusted p value is likely needed than for other data types
ChIP-seq for histone marks	<0.0001	--broad --control <INPUT>	Histone marks are often broadly dispersed without very well defined edges so a broad peak tag is useful but a very low p value helps differentiate between background and data
ATAC-seq	<0.0001	--control <INPUT>	ATAC-seq should show peaks at open chromatin across the genome similarly to histone ChIP-seq data, but with more abrupt peaks, so no broad peak tag is needed

BEDTOOLS

Bedtools allowsus to intersect, merge, and shuffle genomic intervals from multiple files. It accepts genomic file formats such as BAM, BED, GFF/GTF, VCF.

Removing Blacklist regions via **bedtools intersect**. After we call our peaks, to clean up the data we will remove the **BLACKLIST** regions that can be problematic. These regions contain repetitive regions across the genome and almost always are enriched in ChIP-seq data. (**blacklist_hg38.bed** file of blacklist regions in **day09 > data > chip_blacklist** directory).

1.To run **bedtools intersect**, specify **-a** as the file to be filter which is your broadpeak output file. The -a file will be compared against **-b** file which is the blacklist regions. The **-v** parameter will throw out the regions in your peak files that have an overlap with the blacklist regions in **-b**. **>** is to specify the output directory and output file name.




```
echo removing blacklist regions
date
date

bedtools intersect \
 -a ${OUTDIR}/${FILENAME}_peaks.broadPeak \
 -b ${BLACKLIST} \
 -v \
 > ${OUTDIR}/${FILENAME}_peaks_clean.broadPeak

echo macs2 done
date
date
```

2. **sbatch** and run your script. If you want to change the filename input you can do so as show in step 6.

3. Pull the 3 files into IGV to see the different regions and how intersect works.

Reference: https://bedtools.readthedocs.io/en/latest/content/tools/intersect.html

MEME and TOMTOM

MEME suite is useful for motif-based analysis. In the next session, we will briefly demonstrate how you can use MEME for motif discovery and TOMTOM to compare motifs.

1. Copy (**rsync** or **cp**) the **d9_meme.sbatch** from the sample script folder into your script directory that you made in the previous exercise. Recall to copy the script, the command syntax is **rsync <input><output>** or **cp.**

2. MEME suite takes in a fasta file as input. Our MACS peak output is in a bed file format. We will use **bedtool getfasta** and a reference genome .fa file to convert our peaks coordinate into a fasta format. The first thing we will do in our script is to load the appropriate modules.

```
#################### LOAD MODULES ###################################

module load bedtools/2.25.0
module load meme/5.1.1
```

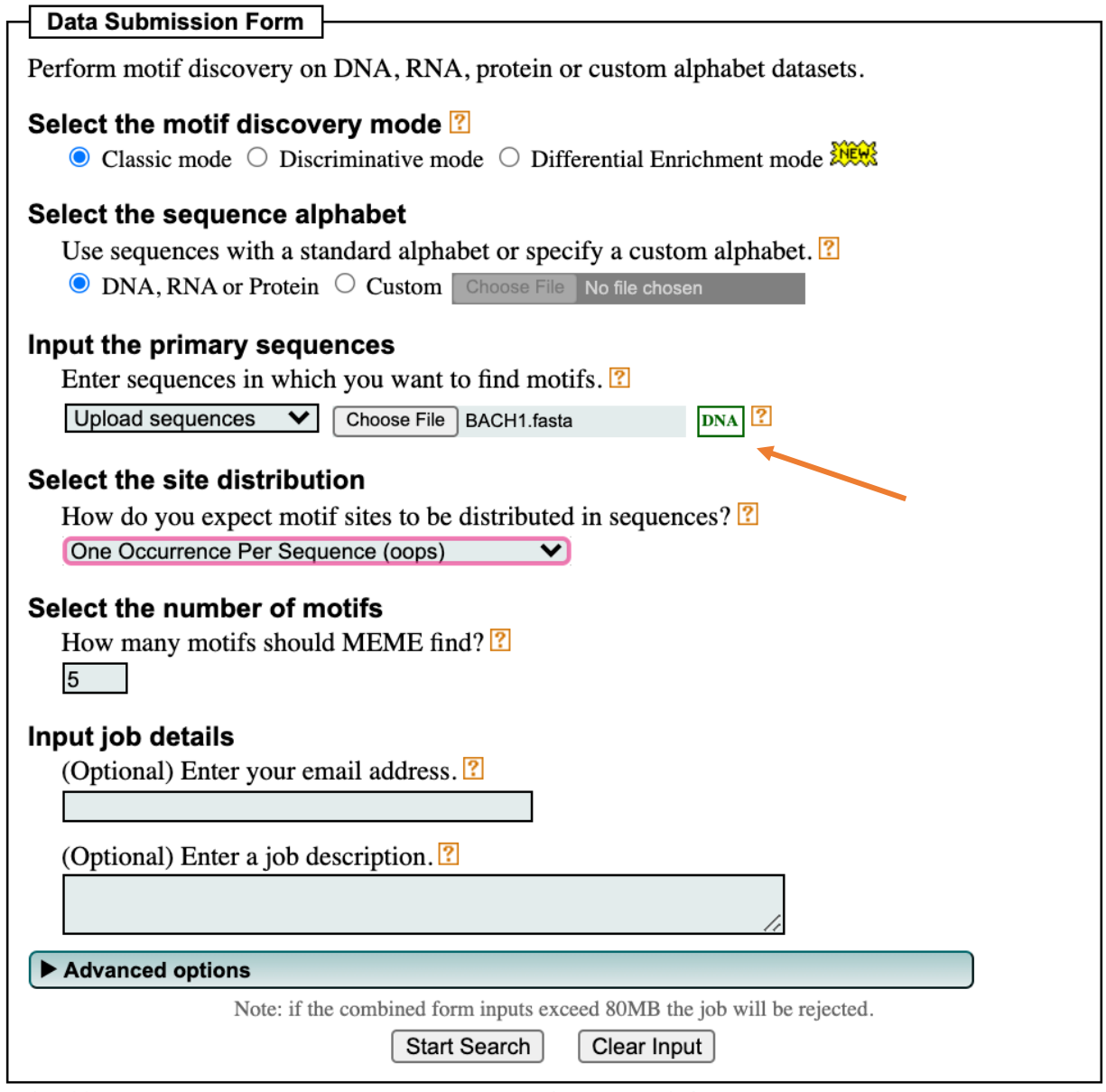
3. Set your in and out directory as we have in the previous exercise. Here your **INDIR** is the path to your macs peak output files. The **OUTDIR** will be for the output of the fasta file and the MEME and TOMTOM output files. Additionally, we will want a reference fasta file denoted below as **hg38.fa** (**hg38.fa** file in **day09 > data > fasta** directory).

```
################## SET VARIABLES ###################################

#INDIR is where your macs output peak files are stored. OUTDIR is where I
 want my output from running meme and tomtom
INDIR='/path/to/macs/peak/files'
OUTDIR='/path/to/output'
mkdir -p ${OUTDIR}

HG38_FASTA='/scratch/Shares/dowell/genomes/hg38/hg38.fa'

FILENAME='BACH1'
```

4. We will use **bedtools getfasta** to convert the peaks to a fasta file to feed into MEME. The command is **bedtools getfasta [OPTIONS] -fi <input FASTA> -bed <BED/GFF/VCF>**

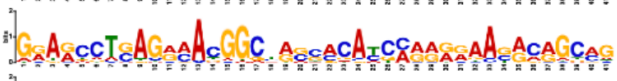
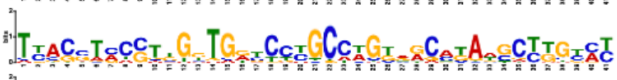
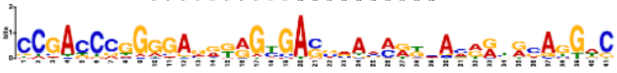

```
#### Get fasta of peak files
echo convert peaks call to fasta format
date
date

bedtools getfasta \
 -fi ${HG38_FASTA} \
 -bed ${OUTDIR}/${FILENAME}_peaks_clean.narrowPeak \
 -fo ${OUTDIR}/${FILENAME}_peaks_clean.fasta
```

5. For running MEME and TOMTOM, you can use command line which I have included a sample script commented out. MEME suite also has a web interface which is what we will use. Rsync your fasta file onto your local drive
6. Upload your fasta file to MEME (https://meme-suite.org/meme/tools/meme) and submit.



7. MEME will return an output file for you. Click on **MEME HTML output**. The output will give you information on the motifs that were discovered along with other information such as the E-value.

DISCOVERED MOTIFS

	Logo	E-value	Sites	Width	More	Submit/Download
1.		9.2e-097	25	41	↧	⇢
2.		1.9e-122	25	41	↧	⇢
3.		8.3e-101	25	41	↧	⇢
4.		2.2e-072	25	29	↧	⇢
5.		6.6e-091	25	41	↧	⇢

Stopped because requested number of motifs (5) found.

8. Push your MEME output to TOMTOM by clicking on the ⇢ under Submit/Download which will open up a new window with available programs. You just have to Start Search to run TOMTOM.

Data Submission Form

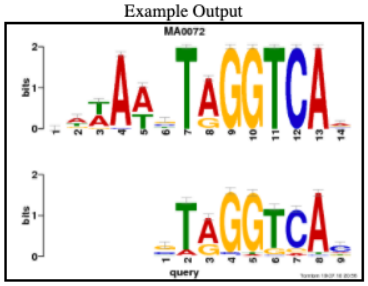
Search one or more motifs against a motif database.

Input query motifs

Enter the motif(s) to compare to known motifs.

Submitted motifs | DNA

Example Output



Select target motifs

Select a motif database or provide motifs to compare with.

Eukaryote DNA | DNA

Vertebrates (In vivo and in silico)

Allow alphabet expansion

Run immediately

Search with one motif (faster queue)

Input job details

(Optional) Enter a job description.

▶ Advanced options

Note: if the combined form inputs exceed 80MB the job will be rejected.

Start Search | Clear Input